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Treatments: DMTS

PRINCIPAL INVESTIGATOR: Matthew Brenner, M.D.

CONTRACTING ORGANIZATION: University of California, Irvine
Irvine, CA 92697-7600

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14. ABSTRACT Lethal rabbit CN poisoning models have been developed in our lab. Animals are monitored for oxy- and deoxy-Hb ratio changes by diffuse optical spectroscopy (DOS) and continuous wave near infrared spectroscopy (CWNIRS), breathed gas parameters, blood gas values and blood pressure during CN treatment and reversal. This year, stability and IM antidote administration studies have been completed using DMTS alone as the CN antidote, one of the 3 novel therapeutic agents under development with our collaborators. DMTS was dissolved in 15% polysorbate solution, and found to be stable for > 1 month. DMTS was successfully administered by IM injection, survival form <15% (controls) to >70%.					
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Brenner, Matthew

Acceleration of Advanced CN Antidote Agents for Mass Exposure Treatments: DMTS

Annual Report, Year 1

Introduction:

The overall goal of this 2 year project is to utilize novel, real-time noninvasive in vivo optical spectroscopic detection methods to investigate potential breakthrough CN poisoning treatment agents, including DMTS alone or with sulfanegen and/or cobinamide, which will be useful for intramuscular (IM) delivery in mass casualty cyanide poisoning settings.

Lethal rabbit models of CN poisoning have been developed in our lab. Animals are monitored in real-time for oxy- and deoxy-Hb ratio changes as determined by diffuse optical spectroscopy (DOS) and continuous wave near infrared spectroscopy (CWNIRS), inhaled and exhaled breath gas exchange parameters, blood gas values and blood pressure during CN treatment and reversal.

In this past year, stability and IM antidote administration studies have been completed in control cyanide poisoned animals, as well as in animals treated using intramuscular injection of dimethyl trisulfate (DMTS) as the CN antidote (one of the 3 novel therapeutic agents under development with our collaborators).

In work this year, DMTS was dissolved in a 15% polysorbate vehicle solution (which was found to be stable for > 1 month). The DMTS/polysorbate agent was successfully administered by intramuscular injection in 12 animals. Survival in the lethal model increased from < 20% in controls to 75% survival in DMTS treated animals. The rate and extent of acute poisoning and reversal in the periphery and CNS region was monitored noninvasively using these optical technologies concurrently with the systemic measurements.

Body:

Scope of Work: The scope of work for this 2-year effort includes the [specific tasks](#) from the original proposal described herein:

1). Investigate the ability of DMTS to be absorbed and reverse cyanide poisoning, and optimize in vivo lipid based delivery in the animal model by intramuscular injection. As part of this process, the contractor will develop noninvasive methods for measuring DMTS absorption/uptake in real-time using DOS. Contractor will utilize novel real-time noninvasive in vivo optical (DOS) detection methods to investigate potential breakthrough treatment agents (DMTS alone or with sulfanegen and/or cobinamide) for mass casualty cyanide poisoning that can be administered intramuscularly (1-4).

Status: Progressing on schedule and successfully for this task.

Progress: Prior to and during the first 3 months of funding of this IAA, a series of dose response experiments using DMTS formulations were carried out. These experiments were successful in determining a dose range over which DMTS appeared to be an effective antidote, but initially, inconsistencies were observed during the series of experiments. In particular, it was noted that older DMTS

solutions were not as effective as new formulations. In conjunction with Drs. Gary Rockwood and Ilona Petricovics, significant loss of DMTS in the polysorbate solution was verified by GC-MS. Studies were initiated to examine the stability of DMTS formulations over time and in various conditions of storage. Experiments revealed that DMTS in polysorbate was stable for 60+ days if stored in airtight, double-sealed vials at 40C. Single-use samples were subsequently provided to us for further experiments, and periodic verification of stability is now carried out by Dr. Petricovics via GC-MS.

Following the completion of stability testing, we were able to complete a series of experiments in the ventilated, lethal rabbit CN model, which verified the efficacy of DMTS injected IM in a lethal, ventilated, cyanide poisoned animal model system. The lethal ventilated model experimental timeline is shown in Figure 1, below, and described in detail in the Supporting Data section below.

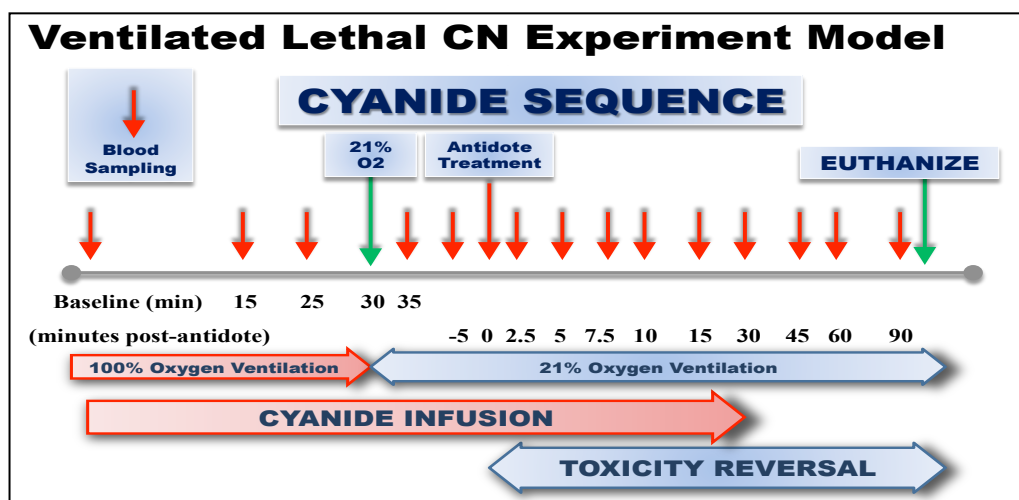


Figure 1: figure 1 shows the study design for lethal cyanide poisoning rabbit model DMTS antidote efficacy investigations. Animals are monitored at baseline. A continuous cyanide infusion is begun. Animals are monitored continuously with CWNIRS and DOS optical technologies, as well as invasive arterial and venous lines, and inhaled and exhaled gas analysis. Animals are given intramuscular antidote injection (or control vehicle injection) when they develop signs of impending lethal CN toxicity (criteria as described in detail in appendix).

Results:

75% (9 of 12) of the animals treated with DMTS survived the experimental protocol to the 90-minute post-antidote timepoint. This is in comparison to a 20% survival in cyanide-poisoned animals treated with placebo injection.

2. Evaluate the effectiveness of combined DMTS with cobinamide and sulfanegen.

Experiments to investigate the effectiveness of DMTS when combined with cobinamide and sulfanegen have begun. The submitted project timeline included the completion of these experiments as a Year 2 task. Thus far, the combination therapy appears to be highly effective. Reduced doses of cobinamide and DMTS are showing effectiveness in lethal cyanide poisoning reversal. Studies are ongoing and will be completed on schedule by the end of year two.

3. Assess the effects of these novel antidote agents on CNS region oxygenation and CNS metabolic response using noninvasive/minimally invasive functional optical brain monitoring and imaging technologies.

Continuous wave near infrared spectroscopy (CWNIRS) has been used in all DMTS experiments to date to follow and compare the oxy and deoxyhemoglobin changes and time course in response to CN poisoning and antidote treatment in the brain region as compared to muscle tissue. Figure 2 shows the results of a control experiment, in which animals received cyanide infusion, but no antidote. Comparing CWNIRS plots of oxy and deoxyhemoglobin from the CNS and muscle regions, marked differences are seen in the physiologic effects of cyanide poisoning in CNS regions compared to periphery. As cyanide poisoning progresses, cyanide inhibits cytochrome C oxidase, blocking aerobic metabolism and the ability of tissues to extract oxygen from the circulating blood stream. This is manifested by increases in oxyhemoglobin (red curves) and decreases in deoxyhemoglobin (blue curves). Terminally, cardiovascular collapse ensues and oxyhemoglobin plummets in the final stages (figure 2). Because of the high blood flow, autoregulation, high metabolic needs, and inability to utilize anaerobic metabolism, the CNS changes are more profound and occur much more rapidly than the peripheral muscle effects.

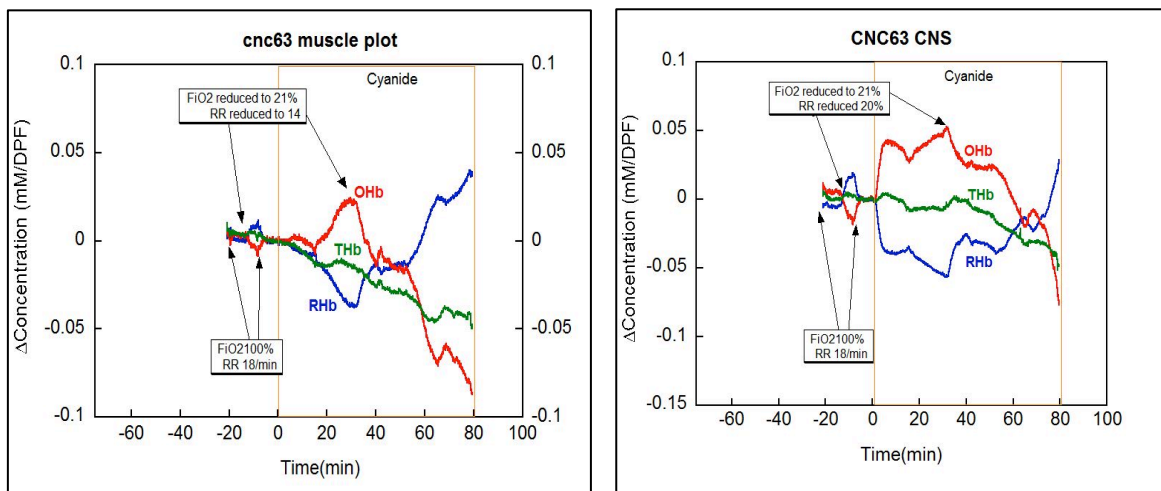


Figure 2. Lethal ventilated cyanide experiment control saline treatment, showing the CWNIRS measured oxy hemoglobin (red, OHb), deoxy hemoglobin (blue, RHb), and green, total hemoglobin during cyanide infusion and saline injection. Saline, given at 30 mins of NaCN infusion, did not reverse the effects of fatal CN poisoning. The CNS/brain region typically shows the hemoglobin effects of cyanide poisoning earlier than the peripheral muscle tissue.

In contrast, when animals are given IM DMTS antidote injection as shown in figure 3, the oxyhemoglobin and deoxyhemoglobin changes are blunted, and the terminal deterioration is prevented.

Figure 3 illustrates the effects of reversal of lethal cyanide poisoning with IM injection of DMTS as monitored noninvasively using DOS and CWNIRS in the

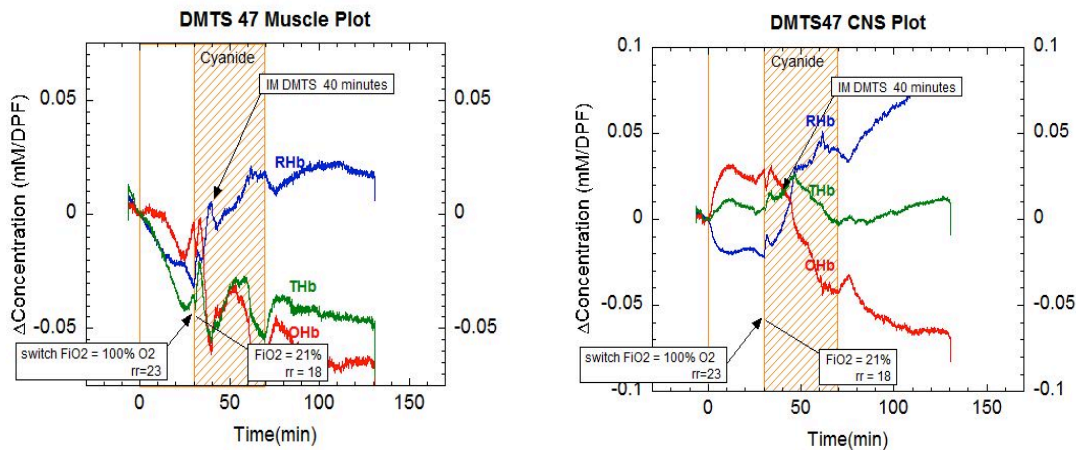


Figure 3: in this figure, animals received continuous cyanide infusion, which results in inability of tissues to extract oxygen. This is manifested by the rise in CNS oxyhemoglobin and decrease in deoxyhemoglobin (right-sided figure). When IM DMTS is administered at 40 minutes into the cyanide infusion, these changes are rapidly reversed and lethality is prevented. In the peripheral muscle region, a decrease in total hemoglobin (green line) is seen that is not present in the CNS due to autoregulation. This decrease in total hemoglobin (secondary to reduced muscle blood flow) accounts in part for the lack of rise of oxyhemoglobin.

peripheral muscle and CNS regions.

These studies confirm the capabilities of DOS and CWNIRS to noninvasively monitor the progression of cyanide poisoning and reversal and effectiveness of antidote therapy.

4. Use a mechanistic research approach to identify targets for therapeutic development using novel in vivo optical signal detection approaches.

This task is part of the overall direction of our laboratory and the collaborative IAA/CounterACT research programs. We continue to examine various metabolic

parameters including oxy and deoxyhemoglobin, inhaled and exhaled gas exchange (anaerobic threshold), hemodynamics, and optically determine to cytochrome C oxidase redox state potential to understand the mechanisms of cyanide poisoning on a physiologic level in vivo. Work is ongoing to evaluate the optical signal information from all DMTS experiments and is continuing with combined therapy antidote administration in year two. Thus far, work has demonstrated that cytochrome C oxidase redox state signals can be determined in vivo (independent from oxyhemoglobin and myoglobin optical cross talk signals), and that significant reduction in cytochrome C oxidase occurs before respiratory exchange ratios increase and anaerobic metabolism ensues. Reversal of cyanide poisoning results in return of respiratory exchange ratios and improvement in cytochrome C oxidase redox state.

These findings will be important in understanding metabolic agent poisoning, providing ongoing means for quantitative determination of treatment efficacy and resuscitation.

5. Perform creation and validation of animal models of chemical effects on humans using novel rabbit poisoning models.

No animal model can perfectly reproduce the human response. Furthermore, human CN exposure scenarios will differ widely. Therefore, numerous animal models must be developed to answer a range of aspects of exposures and treatments. The importance of this issue is further compounded by the fact that human efficacy testing cannot be performed prior to FDA approval for CN antidotes. However, fortunately for metabolic poisons that affect very basic aerobic metabolism pathways, substantial similarities do exist between mammalian species and humans. We have developed a number of rabbit model variations including sublethal models, lethal models, ventilated models, nonventilated models, and IV oral cyanide exposure models. We have administered antidotes via intramuscular injection, inhalation, and intraosseous routes. These animal models are all available for ongoing testing of the novel candidate antidotes alone and in combination as described in the proposal. Work is ongoing.

6. Perform development of preliminary proof of principal data on the efficacy of candidate therapeutics with potential treatment breakthrough agents DMTS, sulfanegen, and cobinamide.

Experiments have been completed that demonstrate the efficacy of each of these agents by intramuscular administration as single agents in the rabbit model. In year two of this proposal, we will study combination therapy using DMTS with cobinamide and DMTS with sulfanegen. Initial studies have demonstrated evidence of effectiveness of DMTS with cobinamide. These studies will be completed in year two as scheduled. Studies of sulfanegen with DMTS will be initiated and completed in year two.

7. Perform development of novel optical methods for determining the rate of antidote

absorption in vivo in brain and peripheral regions using diffuse optical spectroscopy and functional optical monitoring.

Continuous wave near infrared spectroscopy is used in all rabbit experiments to examine the changes in oxy and deoxyhemoglobin ratios in brain and muscle tissue. A comparison of the responses of brain versus muscle has demonstrated increased sensitivity of the brain to cyanide poisoning and reversal in comparison to periphery. This is likely secondary to the multiple factors in the CNS described above including metabolic requirements, inability of CNS to utilize anaerobic pathways, high blood flow rates, and autoregulation.

Key Research Accomplishments:

Task 1:

- Developed stabilized formulations of DMTS polysorbate and methods for storage and transport.
- Demonstrated effectiveness of intramuscular injection of DMTS in reversing lethal cyanide poisoning in the rabbit model.
- Utilized DOS and CWNIRS concurrently with systemic monitoring to determine rates and extent of reversal of cyanide poisoning.

Task 2:

- Initial studies are showing effectiveness of combined therapy with DMTS and cobinamide. Studies are ongoing and will be completed on schedule by the end of year two.

Task 3:

- The CNS and peripheral effects of cyanide poisoning were shown using DOS and CWNIRS. These studies confirm the capabilities of DOS and CWNIRS to noninvasively monitor the progression of cyanide poisoning and reversal and effectiveness of antidote therapy.

Task 4:

- These studies are ongoing. Reversal of cyanide poisoning results in return of respiratory exchange ratios and improvement in cytochrome C oxidase redox state. These findings will be important in understanding metabolic agent poisoning, providing ongoing means for quantitative determination of treatment efficacy and resuscitation.

Task 5:

- We have developed a number of rabbit model variations including sublethal models, lethal models, ventilated models, nonventilated models and oral cyanide exposure models. We have administered antidotes via intramuscular injection, inhalation, and intraosseous routes. These animal models are all available for ongoing testing of the novel candidate antidotes alone and in combination as described in the proposal. Work is ongoing.

Task 6:

- Experiments have been completed that demonstrate the efficacy of each of these agents by intramuscular administration as single agents in the rabbit

model. In year two of this proposal, we will study combination therapy using DMTS with cobinamide and DMTS with sulfanegen

Task 7:

- Ongoing. Differences between CNS and peripheral responses have been observed and described using DOS and CWNIRS technologies.

Deliverables:

1. Demonstrate improved outcome in CN poisoning with DMTS.

Complete Groups 1-3 studies with these endpoints and success criteria:

Endpoints: Primary 1) Survival. Secondary: 1) systemic blood pressure improvement, 2) tissue CcO redox state and systemic gas exchange/hemodynamic improvement.

Success Criteria: Primary: 1) improved survival.

Secondary: 1) statistically significant increase in blood pressure in hypotensive poisoned animals, 2) return of cytochrome c oxidase redox states towards pre-poisoned baseline with gas exchange measures showing improved respiratory quotient.

The primary endpoints of success criteria have been met for DMTS intramuscular treatment of lethal cyanide poisoning in the animal model. Statistically and clinically significant increase in survival from 15% to greater than 70% has been obtained compared to control cyanide poisoned and treated animals. With these dramatic improvements in survival, all of the secondary criteria have also been demonstrated (no terminal blood pressure drops, metabolic, or DOS/CWNIRS terminal phase changes in the surviving treatment group animals that were seen in all of the expiring control group animals).

Work is ongoing for combined therapy animals in year two that will be treated with multiple antidote agents simultaneously.

All work is proceeding on schedule and all tasks have been successfully completed thus far according to the initial proposed timeline.

Reportable Outcomes: (pubs, abstracts, patents, etc.)

These results have been presented at the CounterACT meeting in Washington DC June, 2013. Manuscripts are being prepared at this time. Publication in open meetings and journals is being held (at the request of ICD) until patent rights issues have been cleared by the Department of Defense/ICD allowing us to release findings into the public domain.

Conclusions:

Overall, the work is progressing successfully, and on schedule. We have made a number of advances in demonstration of intramuscular DMTS efficacy in lethal rabbit models of cyanide poisoning. Year two of this proposal focuses on initiation of studies of the effectiveness of combined antidote therapy with DMTS and cobinamide or sulfanegen.

This continued work should help lead to development of effective mass casualty cyanide antidotes and platforms for assessment of effectiveness of antidote therapies for metabolic agents.

We are on schedule to complete all work as per the initial IAA proposal.

References: N/A

Appendices: N/A

Supporting Data:

Detailed procedure for the ventilated, lethal CN rabbit model:

All animals are anesthetized, intubated, and femoral venous and arterial lines placed. After the placement of the femoral catheters, baseline measurements (venous and arterial blood gases, systemic blood pressures, heart rate and SpO₂ values) are taken. The non-invasive DOS (over muscle) and CWNIRS (1 on muscle, 1 over the brain region) monitors are placed. In some animals, the Botvinick microlactate sensor is introduced into the dermal layer of the animal using a 26g needle to provide lactate level measurements. During baseline measurements, the ventilator settings are maintained at a typical respiratory rate of 20 breaths/min, with the inhalation gas input of 100% oxygen.

A pediatric pressure and gas sensor of a GE/Datex Ohmeda S5 (S5) Anesthesia Monitor (with metabolic module, M-COVX) is placed between the "Y" of the ventilator tubing and ET tube connector. After optical measuring devices are connected, the rate of IV anesthesia is increased from .17 to .19 cc/min at least 10 minutes of metabolic data are collected using the S5 equipped with the pediatric flow/pressure pneumotach (data is recorded once a minute on spreadsheet). The S5 can measure oxygen consumption (VO₂) in mls/min, carbon dioxide production (VCO₂) in mls/min, respiratory rate (rr) in breaths /min, inhaled fraction of oxygen (FiO₂) and CO₂ (FiCO₂), end tidal fraction of oxygen (FiO₂ – FetO₂) and CO₂ (FetCO₂), inhaled and exhaled minute volumes (mls/min). After baseline values are taken by the DOS, CWNIRS and S5 with the inhaled fraction of oxygen set to 100% (the S5 device cannot calculate VO₂ when inhaled oxygen is set to 100%), the inhaled oxygen delivered from the ventilator is reduced to 21% (subject breathing room air). Measurements are recorded from the S5 every minute for roughly 10 minutes. After 10 minutes of breathing room air, the ventilator is reset to 100% oxygen and cyanide infusion begins after the effects of inhaling room air are reversed.

Cyanide Infusion:

20 mg of sodium cyanide dissolved in 60 ml of 0.9% NaCl saline is infused intravenously at .33 mg/min (1cc/min). Cyanide is continuously infused for 30 minutes with 100% oxygen input from the ventilator. During cyanide infusion, measurements from the S5 continue to be collected every minute. Time point measurements, which include arterial and venous blood gases, systemic pressures, heart rate, and SPO₂ are taken at time intervals: 15 min after cyanide infusion has started, 25 minutes, and 35 minutes, and continuous DOS and CWNIRS monitoring occurs. At the 30-minute post cyanide infusion time point, the ventilator inlet source is changed from 100% O₂ to room air, and the ventilator setting is adjusted to 16 breaths per minute. The rabbit is then monitored to determine when the cyanide infusion dose has become lethal and when the experimental treatment should be administered. This determination is made as follows:

The effects of cyanide toxicity can be observed in real time, and include a reduction in blood pressure, oxygen consumption, CO₂ production (as well as the fraction of exhaled O₂ and CO₂), and the CWNIRS curves that show changes in the ratios of deoxy HGb and oxy HGb in real time.

The “minus 5 minute” (5 minutes before IM or IV cyanide antidote injection) time point is determined by a reduction of V_{O2} clearly over 50% from baseline, a significant reduction (>20% from baseline) VCO₂, a fall in mean blood pressure 40% from baseline, and the DOS and CWNIRS indicating an acceleration of the effects of cyanide poisoning (increased slope changes in deoxy HGb and oxy HGb). After the “minus 5 minute” blood samples are taken, the experimental treatments are prepared. At time point “0”, each animal will then receive the cyanide reversing drug treatment DMTS (or vehicle control). The treatment is administered via intramuscular injection into the (pectoral muscle), via intraosseous injection into the tibia, or nebulized using a DeVilbiss ultrasonic nebulizer. Following the injections, blood pressures are monitored every minute and noted for the first ten minutes. Cyanide level and blood gas samples are taken at times 0 min, 2.5 min, 5 min, 7.5 min, 10 min, 15 min, 30 min, 45 min, 60 min, and 90 minutes post treatment. Cyanide infusion continues for 30 minutes post treatment. Time point measurements, including arterial and venous blood gas, heart rate, blood pressure and SPO₂ are also taken at time 5 min, 15 min, 30 min, 45 min, 60 min, and 90 minutes. At the 90-minute time point, final measurements are taken, and the rabbit is euthanized. If at any time during the experiment, the systolic blood pressure falls to 75% below the baseline value, or 25mmHg systolic, (this occurs in > 80% of the control animals receiving no antidote treatment), the procedure will be considered “lethal”, the experiment will be stopped, and the animal are euthanized immediately.